The influence of some features of *in vitro* cultivation on direct germination, callusogenesis and morphogenesis in the culture of mature barley embryos

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The influence of light, sterilization conditions and some components of the nutrient medium on direct sprouting, callusogenesis and morphogenesis in the culture of mature barley embryos was studied.

***Keywor**ds: Barley, in vitro, mature embryos, cultivation conditions, direct sprouting, callusogenesis, morfogenesis*

INTRODUCTION

Immature and mature embryos are traditionally used as explants for production of cellular tissues of cereal crops. Numerous experiments reveal that both mature and immature embryos of cereal plants under in vitro cultivation conditions tend to germinate (Ziebur et al, 1950; Norstog, Klein R.,1972; Campenot et al., 1992; Popelka, Altpeter, 2001; Birsin, Ozgen, 2004; Juravlev, Omelko, 2008; Zobova et al., 2011; Nikitina, Khlebova, 2014), and this considerably complicates production of embryogenic callus tissues from them. Provision of conditions preventing direct germination of embryos and acceptable for obtaining morphogenic callus will allow to increase regeneration potential of barley, which is one of the most compound objects for in vitro cultivation. Therefore, the purpose of this work is to study some features of cultivation conditions, which assume prevention of direct germination of mature barley embryos, favorable for callusogenesis and morphogenesis.

MATERIAL AND METHODS

As the objects of the research Azerbaijani barley varieties, such as Nakhichevandany, Bakharly, Jalilabad – 19, Karabakh - 22, Dayanatly, Gudratly – 48 were used. Varieties differed in degrees of resistance to certain climatic conditions and some diseases had a different number of rows in the ear. Before isolating the embryos, sterilization of the grain was carried out in two versions:

1. version – 15-minute sterilization in 50% sulfuric acid, 5-minute sterilization in 70% ethanol and 18-minute sterilization in 5% sodium hypochlorite;
2. version: 5-minute sterilization in 70% ethanol and 18-minute sterilization in 5% sodium hypochlorite.

After each change of the sterilizing solution, the grains were washed 3-4 times with distilled water.

Mature embryos were extracted from the seeds and planted on the Murashige-Skoog medium supplemented with the hydrolyzate of the casein and a 50-fold increased amount of CuSO4 (Dahleen, 1995). For the induction of callusogenesis, two versions of the medium differing in the phytohormonal composition were used: in the first version of the medium 2.5 mg/l of 2,4-D + 0,5 mg/l of BAP and in the second version 2.0 mg/l of 2.4-D + 0.1 mg/l of kinetin were added. Cultivation was carried out in dark and in light at a temperature of 26°C.

Morphogenesis was stimulated on nutrient media with the same composition and carried out in light at a temperature of 26°C.

RESULTS AND DISCUSSION

Our previous studies (Asadova, 2015; 2017) revealed that immature and mature embryos of the barley varieties on mediums for induction of callusogenesis tended to germinate when they were cultivated in the dark. In order to determine the conditions under which germination of embryos would be lower than in the control variant, observations were started considering also the sterilization options, since there are some records arguing on possible influence of sterilizing agents on the germination of embryos and callusogenesis (Popelka, Altpeter, 2001**;** Yu et al., 2008). Hydrolyzate of casein and 50-fold increased copper sulfate were added to the cultural medium as additional nutrients, which are also used in the form of agents inhibiting germination of embryos. The influence of these factors on the processes of callusogenesis and morphogenesis was considered. In addition, the effect of various compositions of phytohormones on these processes was observed (Mardamshin, Mustafina, 2001; Przetakiewicz, 2003; Soboleva, 2005; Juravlev, Omelko, 2008). Keeping embryos in dark and in light also was aimed at revealing the optimal conditions for cultivation.

Observations revealed that in the first variant of sterilization, where sulfuric acid was additionally used, the incidence of germination was smaller compared to the second variant used as a control. In addition, no cases of explant infection were observed. The use of casein hydrolyzate and copper sulfate at this stage of the experiment was not effective, as there were no significant differences by the number of germinating embryos compared to the control variant. The varietal dependence is recorded. Under these conditions germs of Bakharly variety were least prone to germination while germs of Jalilabad – 19 variety most often germinated. Germination of explants Jalilabad-19 was observed in both variants of the medium with different phytohormonal composition. However, for all varieties, a nutrient medium containing 2,4-D and kinetin had an advantage not only in cases of germination of germs, but also in the induction of callusogenesis. Moreover, if the embryo germination was observed, it was accompanied by callusogenesis (Fig. 1), callus tissue continued to proliferate with subsequent truncation of the seedling.

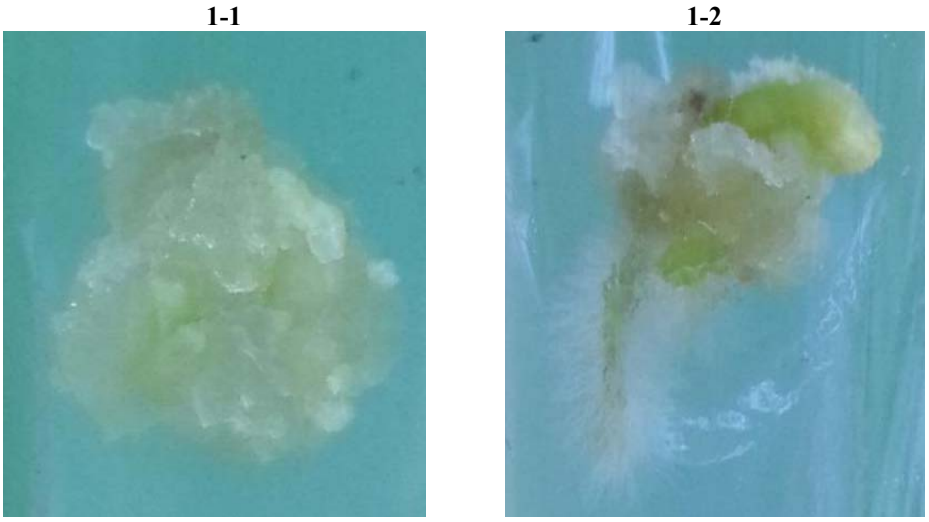

Fig. 1. Callusogenesis (1-1) with simultaneous germination of the embryo (1-2).

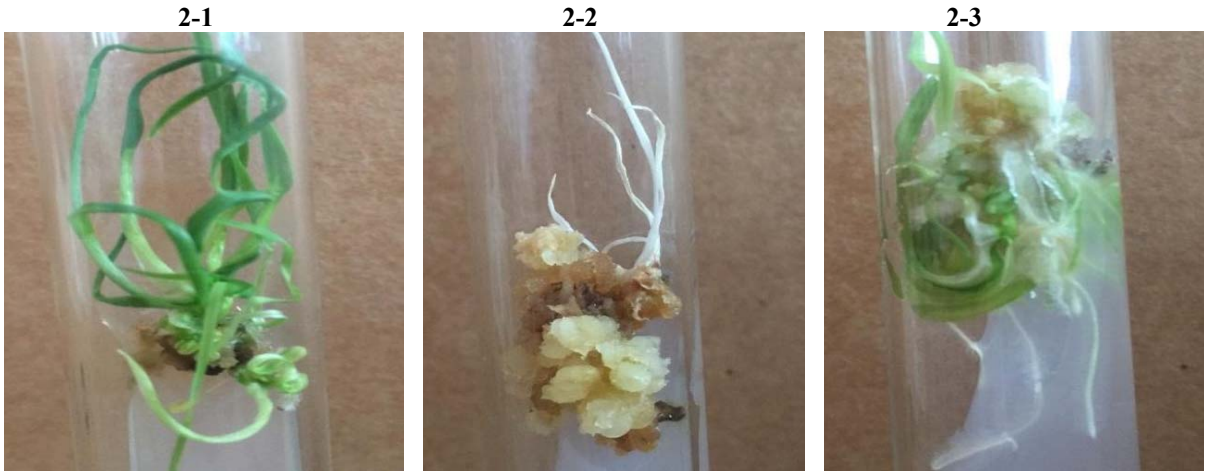

Fig. 2. Gemmagenesis (1-1), rhizogenesis (1-2) and gemmarhizogenesis (1-3) in morphogenic callus.

Cultivation of explants in light and dark had no significant visual effect on proliferation and formation of morphogenic callus. The difference was observed only in the color of callus, which had a greenish tinge due to cultivation in light. Both light and dark cultivation were carried out in an

During induction of callusogenesis the best results were observed in varieties Nakhichevandany and Dayanatly. Morphogenic calluses were obtained in both variants of the experiment with different phytohormonal composition, wherein the processes of embryogenesis, rhizogenesis, gemmagenesis, and gemmarhizogenesis were observed (Fig. 2).

A different morphogenetic reaction may be associated with the features of the endogenous hormonal regulation of each of the studied varieties (Carnes. Wright, 1988; Mardamshin, Mustafina, 2001; Przetakiewicz, 2003; Juravlev, Omelko, 2008). Influence of the modified concentrations of inorganic components on this process is also possible (Becher, 1992; Nuutila, 2000; Dahleen, Bregitzer, 2002; Chauhan, Kothari, 2004; Demenko, 2010). Presence of a modified concentration of inorganic components in the medium, in particular copper, may be facilitates activation of regeneration processes (Dahleen, 1995; Bregitzer, 1998; Nuutila, 2000; Tahiliani, Kothari, 2004; Kothari et al., 2004; Yu et al., 2008). The studies of these authors showed that in order to obtain successful in vitro reproduction of valuable varieties, it is necessary artificial climate chamber at the same temperature. Probably, a positive result is possible with a short cultivation of explants under conditions of elevated temperature (Pestana, 1999; Popelka, Altpeter, 2001; Zobova et al., 2011; Nikitina, Khlebova, 2014).

to develop regeneration protocols specific for each individual genotype. Particularly with regard to barley genotypes, distinguished by a set of economically valuable traits, since such plants are characterized as genotypes with low morphogenetic potential and small regenerative capacity (Dahleen, 1995; Dahleen, Bregitzer, 2002; Bakulina, 2016). In our experiment, all the varieties used have economically valuable characteristics, especially Gudratli-48 variety, which was recently created at the Research Institute of Crop Husbandry. It exceeds all standard varieties of this type in terms of economically valuable characteristics and biological properties (Mammadov et al., 2010).

In general, it can be said that the inclusion in the sterilization protocol of concentrated sulfuric acid has had a positive effect in preventing germination of mature embryos and obtaining material maximally decontaminated from infections. The hydrolyzate of casein in our experiment did not yield the expected result, germination frequency of the embryos did not differ from the control variant. As expected (Popelka, Altpeter, 2001**;** Yu et al., 2008), use of increased copper sulfate concentration stimulated

the processes of morphogenesis and regeneration. The best indicators of which at this stage of the experiments were obtained for the varieties Nakhichevandany and Dayanatly.

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Bəzi *in vitro* kultuvasiya rejimlərinin arpanın yetişmiş rüşeym kulturasında birbaşa cücərmə, kallusogenez və morfogenez proseslərinə təsiri

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İşıqlanma, sterilizasiya rejimi və qida mühitinin bəzi componentlərinin arpanın yetişmiş rüşeym kulturasında birbaşa cücərmə, kallusogenez və morfogenez proseslərinə təsiri öyrənilmişdir.

***Açar sözlər**: Arpa, in vitro, yetişmiş rüşeym, kultivasiya rejimi, birbaşa cücərmə, kallusogenez, morfogenez*